Surveillance of Acute Infectious Respiratory Disease in Select Populations: US Embassy Employees

WRAIR Study No. 1514A

Division of Viral Diseases
Walter Reed Army Institute of Research

Version 7- 15 December 2008

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Version 7

15 Dec 2008

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1.General Information

1.1. Protocol Title: Surveillance of Acute Respiratory Disease in Select Populations: US Embassy Employees.

1.2. Version Date/ WRAIR Study Number: 15 December 2008/1514A

1.3. Sponsor: Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD 20910-7500. Funding provided by: Department of Defense Global Emerging Infections Surveillance and Reporting System (DoD-GEIS), Silver Spring, MD 20910-7500 (Funding number: I0070_09_WR, I0090_09_WR).

1.4.Principal Investigator:

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1.4.1.Roles and Responsibilities of the Principal Investigator: Has overall responsibility for the planning and execution of all aspects of the protocol, including compliance with all relevant Department of Defense (DoD) and WRAIR policies and procedures and Army regulations. These activities include by are not limited to: report and document adverse events, report changes and unanticipated problems, report any change of investigators, prepare continuing review reports at intervals designated by the WRAIR Institutional Review Board (IRB) and a final report.

1.5. Associate Investigators:

1.5.1. Robert Kuschner, COL, MC, DVD, WRAIR, Silver Spring, MD 20910, TEL: (301) 319-9012 FAX: (301) 319-9661, Email: robert.kuschner@us.army.mil

1.5.2. Julia Lynch, COL, MC, DVD, WRAIR, Silver Spring, MD 20910. TEL: (301)319-9434; FAX: (301)319-9661. Email: Julia.lynch@us.army.mil.

1.5.3. John J. Keyes III, MD, MPH, FAACP, M/Med/DASHO/Director of Operation, Office of Medical Services, US Department of State, SA-1, Room 2210, 2401 E Street NW, Washington, DC 20522-0101. TEL: (202)663-1518; FAX: (202) 663-3673; Email: KeyesJJ@state.gov.

1.5.4. Roles and Responsibilities of Associate Investigators: Support the Principal Investigator in planning and execution aspects of the protocol.

1.6. Location of Study

1.6.1. Surveillance: Selected US Embassy medical facilities operated by the US Department of State (DoS) worldwide. Locations in Appendix 5.

1.6.2. Laboratory: DVD, WRAIR, 503 Robert Grant Ave., Silver Spring, MD 20910-7500.

1.7. Time Required to Complete Study: 5 Years (Start approximately 1 November 2008, Complete approximately 31 October 2013).

1.8. Study Overview: This is a surveillance study of the causes of acute febrile respiratory disease in personnel located at various US Embassy facilities operated by the DoS worldwide. Personnel who self-report with influenza-like illness (ILI) symptoms will be asked to complete a brief questionnaire about their illness and to provide a respiratory specimen that will be analyzed for a battery of viral and bacterial pathogens in the DVD, WRAIR. Pathogens of special interest, particularly influenza and adenovirus, may undergo full-length genomic sequencing when this information may contribute to our understanding of pathogen epidemiology, the clinical spectrum of disease, drug resistance, or have the potential to identify influenza vaccine strains.

The US Embassy sentinel surveillance sites will be important parts of a global network of respiratory disease (DoD-Global Emerging Infections Surveillance and Response System (GEIS) that will enhance the preparedness of the U.S. for annual, pandemic, and avian influenza as well as outbreaks of other emerging pathogens. This study will provide important information about influenza and other respiratory pathogens in a select population of individuals who regularly travel to and from the continental United States (CONUS) and the surveillance sites. The etiologic causes of respiratory disease in this population have not been previously determined. Individuals located at these sites also are potentially exposed to a variety of unknown pathogens that cause ILI not captured in classical assays.

In addition to the surveillance component of the protocol as outlined above, there will be a research component involving selected respiratory specimens that have been obtained in this protocol. These specimens will be utilized to support assay development and validation at DVD, WRAIR. Specimens will be selected so as to ensure adequate representation of known pathogens (e.g., influenza A, Influenza B, Parainfluenza 1-3, RSV, Adenovirus, etc.) as well as negative specimens, which are required to assess the specificity of new assays. However, the actual test validation methods are not the subject of this protocol. Instead, separate validation protocols will be drafted, as appropriate, for each assay.

The respiratory samples will be used only for surveillance research purposes, and no commercial use will result from the use of these specimens.

2. Background Information

2.1. Investigational Product: The TessArae®, Roche 454 pyrosequencer, and the SeeGene system are investigational devices. The CDC influenza RT-PCR assays and the Luminex system are FDA licensed devices. See section 3.2 for a more complete description of these devices.

2.2. Findings from clinical and non clinical studies that have relevance to this study: Human and Avian influenza (AI) and other emerging respiratory infections are important threats to the United States. In response to a Presidential directive in 1996 to address this threat, the DoD established GEIS. GEIS has established a global surveillance network that monitors host nation populations in areas where disease epidemiology was previously unknown or inadequately characterized. However, important gaps persist[1]. While GEIS surveillance includes some deployed U.S. military and civilian personnel, many individuals who work outside the U.S. and return on a periodic basis are not currently captured in the network[2]. These individuals may be at risk of acquiring and transmitting the next pandemic-whether it be influenza or another pathogen. The US DoS oversees all US Embassies located globally. Embassy personnel work in these locations on a temporary basis, travel frequently between their respective country and the US, and also host visitors (e.g., scientists, students, tourists, etc.). Because of their location, personnel in these facilities come in frequent contact with local populations that are carriers of and transmit unknown and emerging pathogens capable of causing ILI. Currently, there is no systematic effort to determine the causes of respiratory disease in this important population. This study will evaluate the contribution of influenza, avian influenza, and other routine and emerging pathogens to acute respiratory disease in employees and visitors at selected US Embassy facilities globally. This protocol currently seeks approval to conduct surveillance of the US Embassy facilities as listed in Appendix 5; these sites were chosen because all are readily accessible by courier and all have either Regional Medical Technologists (RMTs) or Foreign Service Regional Medical Officers (FSRMOs) or Foreign Service Health Practitioners (FSHPs) on-site.

2.3. Potential risks and benefits to the volunteers: Collection of the respiratory specimens may involve temporary discomfort and irritation of the pharynx or nares. There is a risk of breach of confidentiality. There is no direct benefit to the volunteer.

2.4. Description of the population to be studied: The surveillance portion of the study will be conducted in US Embassy facilities operated by the US DoS worldwide. US Embassy personnel of all ages (newborn to 65) and visitors, which may include foreign nationals, to these facilities will be asked to enroll if they self-report, or, in the case of children, their legal guardians bring them to the health care facility with symptoms of acute respiratory disease, including cough, runny nose, stuffy nose, sore throat, and shortness of breath with, or without,

fever. To be included in the study, all subjects (and legal guardians, in the case of children) will be required to understand English. Inclusion of children is important, to get an accurate representation of pathogens causing ILI in the study population.

3. Study Design

3.1. Study Objective: This is a surveillance study intended to provide information about the infectious causes of acute respiratory disease in the study population

3.2. Study Methods:

3.2.1. Study Samples and Data: No formal hypothesis will be tested. Descriptive statistics will be used to summarize the aggregate results, the results within each site, and to compare the different sites. It is estimated that approximately 500 subjects will be enrolled in the study during the first year, with 1000 specimens submitted. It will be difficult to estimate the number of cases, because of variations in reported cases through the year, as well as variations in the number of cases Embassy to Embassy. A better estimate will be given after the first year of the study.

3.2.2. Collection of Respiratory Samples and Questionnaire: Respiratory collection kits, questionnaires and instructions will be provided to each site. Respiratory samples will be collected by trained US Embassy staff.

Upon encountering an individual with respiratory illness in the examination room (to facilitate privacy) or equivalent, US Embassy personnel (RMO, FSRMO, FSHP, LES or designee) will describe the study and, if the individual is willing, then provide the informational document to the individual for their review. The subject will have time to ask questions and to consider whether they want to participate. If the individual agrees to participate, the volunteer will complete the questionnaire that is consistent with World Health Organization (WHO) standards for ILI surveillance (Appendix 3).

The designated US Embassy personnel will check off the box on the questionnaire signifying that the informational sheet was given to the volunteer, affix one label (appendix 2) to the document and then will obtain one nasal swab and one throat swab from the volunteer. While the collection of a single throat swab is standard of care, collection of nasal and throat swabs are being done for the purposes of the study; nasal specimens are preferred for recovery of human influenza, while throat specimens are preferred for recovery of avian influenza. If the collection of nasal swabs proves to be too difficult or too uncomfortable for the patient (i.e., children), a throat swab alone will suffice.

Management of the illness will occur outside of the terms of the protocol and will not be affected by the decision of the individual to enroll or not or by the results of the study.

3.2.3. Sample transportation and Storage: Swabs will be inserted into transport media. The tube will be sealed and the labels (appendix 2) attached. When available, samples will be placed without delay in a -70° C freezer. If not immediately available, samples may be kept on ice or in a refrigerator for up to 96 hrs before transfer to the -70° C freezer. Samples will be stored in a controlled, secured environment until shipment to WRAIR. Alternatively, if -70 ° C freezers are not available, special media (e.g., lysis buffer or equivalent) will be used to preserve nucleic acid integrity, but not pathogen viability. Commercial shipping services (e.g., WorldCourier, FedEx, DHL, etc) will be routinely utilized to ship the specimens in accordance with applicable regulatory requirements, including those of the International Air Transport Association (IATA). Alternate methods (i.e., diplomatic pouch) may be utilized if samples cannot be sent with dry ice. Samples, questionnaires and the sample inventory will be shipped at the same time to the following address: DVD, WRAIR 503 Robert Grant Avenue, Building 503, Room 3A04, Silver Spring, MD 20910.

3.2.4. Inprocessing of samples at DVD, WRAIR: On arrival at DVD, WRAIR, the samples are processed (inventoried) and stored by the Compliance Management Unit (CMU) DVD, WRAIR until their release for testing. Media will be maintained at -70^0 C (+/- 10^0 C) upon arrival. Once tested, all of the remaining sample will be stored at DVD, WRAIR under a Repository Protocol (WRAIR 1367) for possible future use in the development of diagnostic tests and other, as yet unknown, investigations.

3.2.5. Laboratory Evaluations of Subject Samples: All lab personnel will observe standard precautions during handling, processing and analysis of these samples, regardless of pathogen content. Once received at WRAIR DVD, samples will undergo total nucleic acid extraction followed by testing for influenza and other respiratory pathogens with nucleic acid-based assays including Luminex® , Seegene, TessArae®, CDC RT-PCR, and the Roche 454 pyrosequencer systems. All samples will be tested initially with individual PCR assays (CDC) for influenza (influenza A, B, H1, H3, H5,) as well as the Luminex® system; initially, most samples will also be analyzed by the TessArae® system, but if results are duplicated from the Luminex® system, the numbers of samples analyzed with this system will be reduced. Only selected samples will be analyzed with the Roche 454 FLX sequencer system. In addition, culture of selected agents will be done with retention of the agent in WRAIR DVD archive. Isolates of potential interest for vaccine development (novel or emergent strains) will be sent to USAFSAM for

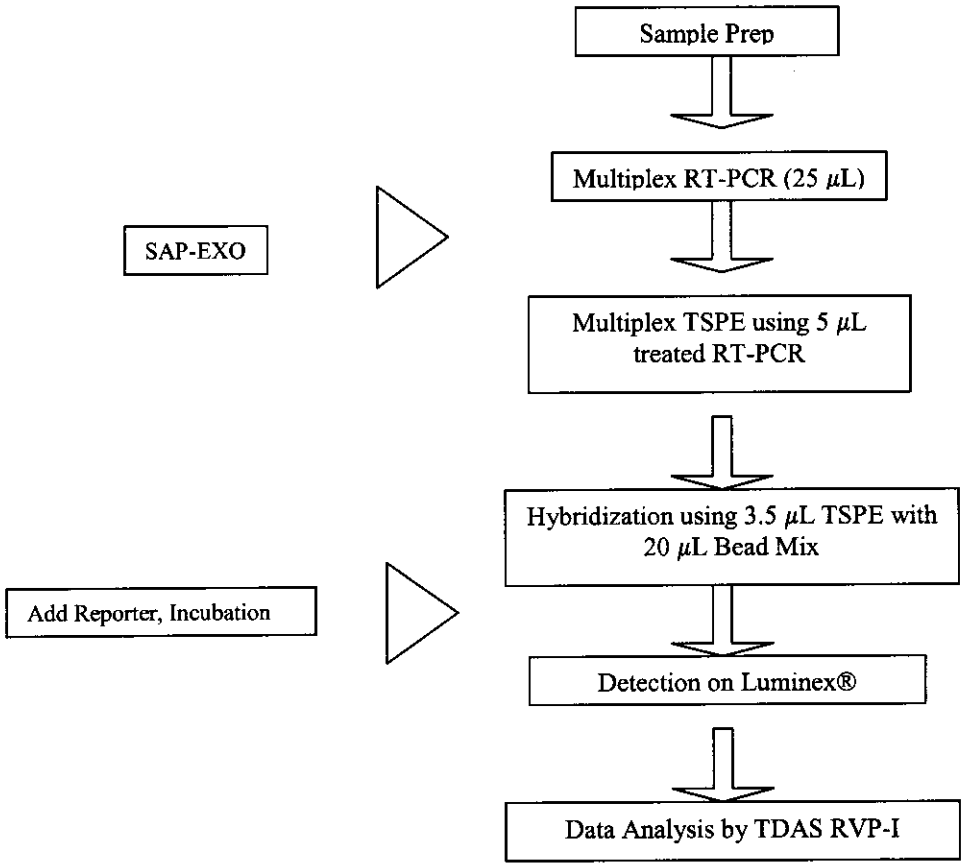
further characterization. Isolates sent to USAFSAM will be maintained at USAFSAM.

3.2.5.1.CDC Influenza PCR Assays: The Centers for Disease Control (CDC) RT-PCR assays for human influenza A and B and subtypes H1, H3 and H5 will be utilized to identify and characterize influenza in collected respiratory specimens. These assays are FDA-cleared. These assays will be conducted per CDC protocol Ref.# I-007-05. This is surveillance.

3.2.5.2.Luminex® Assay system

Luminex® is a multiplex PCR system designed to identify pathogens to the species (and in some cases, subtype) level. It uses a multiplex PCR technology called target-enriched multiplex PCR to simultaneously amplify multiple molecular targets in one reaction. The Luminex® assay system uses the xTAG® Respiratory Viral Panel (RVP) which is a qualitative nucleic acid multiplex test intended for the simultaneous detection of multiple respiratory virus nucleic acids, including Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Synctial Virus Subtype A, Respiratory Syncytial Virus Subtype B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Human Metapneumovirus, Rhinovirus and Adenovirus. This assay is FDA cleared. This is surveillance.

Luminex's xMAP technology is built on flow cytometry, microspheres, lasers, digital signal processing and traditional chemistry. xMAP bioassays incorporate a multiplex PCR technology called target-enriched multiplex PCR to simultaneously amplify multiple molecular targets in one reaction. This provides a means to establish a sensitive and efficient assay system to monitor the emergent respiratory infections caused by numerous known pathogens[3].



3.2.5.3.SeeGene System

The Seeplex RV/PB18 ASE Detection assay is a commercially available diagnostic kit designed to detect simultaneously 13 respiratory viruses and 5 pneumonia bacteria from respiratory specimens. Seeplex is a system based on a multiplexed PCR that uses a proprietary Dual Priming Oligo system, which offers high specificity, reproducibility and sensitivity of amplification of target sequences. The nucleic acids from the clinical specimens are extracted, and the sample is reverse transcribed and amplified by a single PCR. The PCR products are fluorescently labeled, and their presence is detected using capillary electrophoresis. This technique has been used for the diagnosis of several diseases, including respiratory pathogens[4-6]. We will use the Seeplex RV/PB18 ASE Detection assay in parallel with and compare it with the Luminex assay. The assay detects Legionella pneumoniae, Streptococcus pneumoniae, Chlamydophila pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Influenza A, Influenza B, RVS A, RSV B, Parainfluenza 1-3, Coronavirus 229E/NL63 and OC43/HKU1, Rhinovirus, Enterovirus,

Adenovirus, and Bocavirus. The assay is not yet FDA-cleared. This is research.

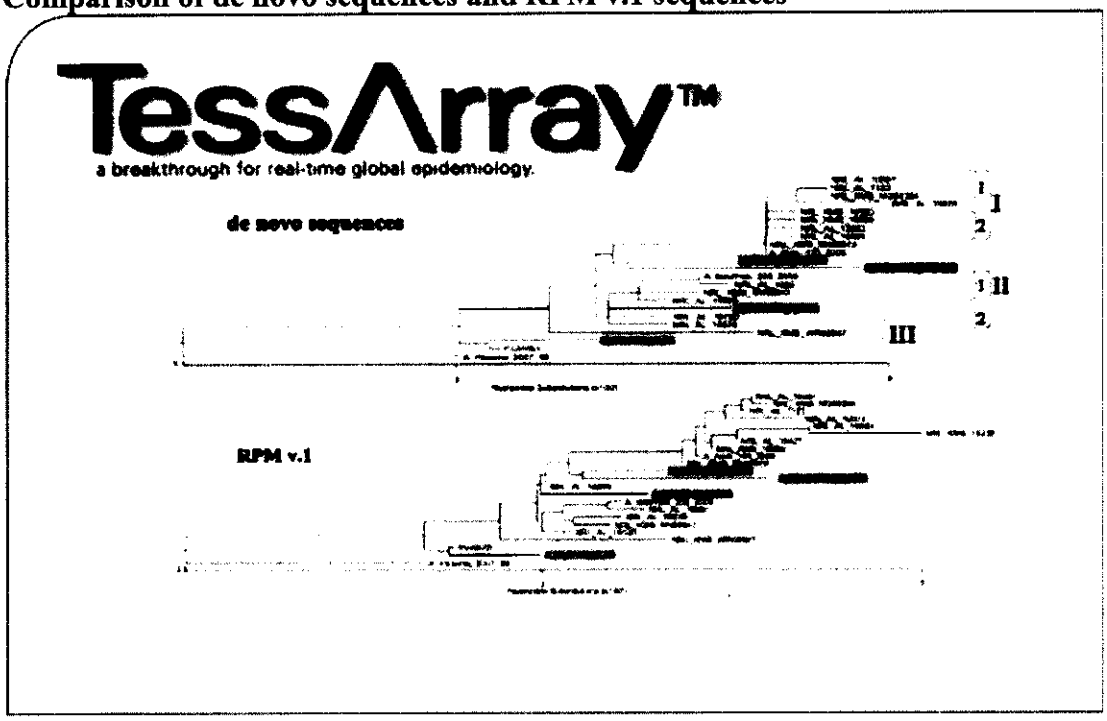
3.2.5.4. TessArae® System

TessArray® is a high density DNA microarray system utilizing the Resequencing Pathogen Microarray version 3.1 (RPM v3.1) with the capability of identifying a larger number of pathogens and also provides partial genome sequence information. Seventy-one different viral and bacterial agents can be assayed for using this platform, simultaneously detecting and identifying these pathogens and providing genus-, species, serotype- and strain- level information. Pathogens detected include influenza A and B (all 16 HA subtypes and all 9 NA subtypes), Parainfluenza 1-4, RSV A and B, adenovirus, Coronavirus, Rhinovirus (27 types), coxsackievirus (5 types), Echovirus (8 types), Metapneumovirus A and B, Variola major, as well as 21 bacterial respiratory pathogens. Advantages of this system include 1) Greater accuracy than hybridization of a pathogen-specific probe (as in PCR); 2) Sequence variations, either naturally occurring or in response to environmental stress (e.g. antibiotic resistance) are also identified, 3) Range of detected pathogens is broadened and 4) The assay has been validated in multiple clinical settings. The parent company, Tessarae, is in discussions with the FDA for review of the RPM chip. This is research.

TessArray® Resequencing Pathogen Microarray (RPM) platforms are high density microarrays based on the Affymetrix® CustomSeq® resequencing microarray platform, using TessArray proprietary methodologies for identifying pathogen gene sequences, and bioinformatics tools for data analysis. TessArray RPM arrays interrogate 117,000 bases of DNA and/or RNA sequence, which allows for very broad analysis of numerous known and unknown pathogen strains. Total time-to result is less than one day.

Sequence-based methodologies, including RPM, provide an additional layer of information beyond presence/absence of the pathogen, which is the nucleotide sequence of the pathogen-specific signature, whether family-, genus-, species, or strain-level identification, so the accuracy (or confidence in the identification) is much greater than hybridization of a pathogen-specific probe (as in PCR). Another major benefit of sequence is that variations in the sequence, either naturally occurring or mutations in response to environmental stress (e.g. antibiotic resistance) are also identified, and still provide useful information about the identity or characteristics of the pathogen. Such sequence variations may reduce hybridization efficiency of a pathogen-specific probe, leading to a false negative or indeterminate result.

Figure 1. Comparison of de novo sequences and RPM v.1 sequences



3.2.5.5.Roche 454 Genome Sequencer FLX System

The Roche 454 FLX pyrosequencer system is capable of providing 1) Full-length high-throughput genomic sequence information, 2) Ability to conduct pathogen discovery investigations in outbreaks of unknown cause, and 3) Focused, partial-length sequencing of genes of interest, such as antiviral resistance genes, with the capability to identify rare resistant mutants (quasi-species) that remain undetected by classical methodologies. Only selected samples will be analyzed with the Roche 454 FLX sequencer system. This is research.

The Genome Sequencer FLX is a complete system including instrumentation, reagents, and software, for high throughput DNA sequencing. The informatics software provides comprehensive de novo assembly of whole genomes as well as variant analysis of amplicons. The reagents provide a turnkey solution from library construction to amplification and sequencing. The supplied informatics software enables de novo assembly, with optional paired end assembly, of genomes up to one hundred million bases, mapping of genomes up to three billion bases and amplicons variant analysis.

The system is capable of expressed sequence tag (EST) sequencing, the identification of small RNAs and transcription binding sites as well

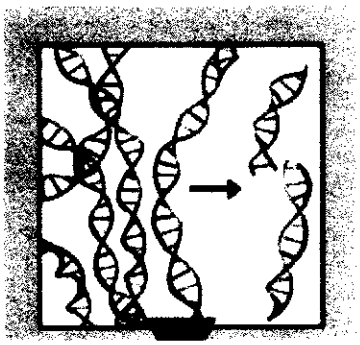
as whole genome sequencing and metagenomics. The complete sequencing workflow of the system comprises four main steps from purified DNA to analyzed results. These basic steps include 1) Generation of a single-stranded template DNA library, 2) Emulsion-based clonal amplification of the library, 3) Data generation via sequencing-by-synthesis, and 4) Data analysis using different bioinformatics tools.

DNA samples are broken into fragments that are between 300 and 500 bases in length. These fragments are then ligated by two short adaptors, which provide primers for both amplification and sequencing of the fragment as well as a biotin tag that immobilizes it onto a streptavidin-coated bead.

Very dilute solutions containing the DNA fragments are used, such that each 28um bead binds only a single DNA fragment. The beads are then emulsified with PCR reagents in water-in-oil microreactors so that clonal amplification can occur. This yields beads with millions of copies of the DNA fragment attached. These beads are then deposited in the PicoTiterPlate device by centrifugation before enzyme beads and incubation mix are added.

The PicoTiterPlate contains over 1.6m wells that are approximately 44um wide and fit one bead per well. Each well has an optical fiber attached to its base, which form an array leading to the CCD camera.

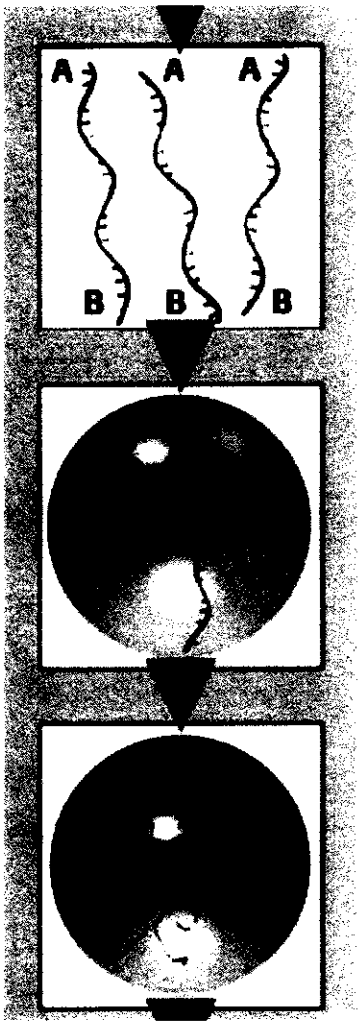
The fluidics system allows nucleotides to be pumped in, in a fixed order. During the nucleotide flow each of the beads is sequenced in parallel, with the polymerase extending the sequencing strand only if the nucleotide is complimentary to the template strand. The addition of one (or more) nucleotides results in a reaction that generates a light signal, which is recorded by the instrument's camera.



Sample Input and Fragmentation

The Genome Sequencer FLX System supports the sequencing of samples from a wide variety of starting materials including genomic DNA, PCR products, BACs, and cDNA. Samples such as genomic DNA and BACs are fractionated into small, 300- to 800-basepair fragments. For smaller samples, such as small non-coding RNA or PCR amplicons, fragmentation is not required. Instead, short PCR products amplified using Genome Sequencer fusion primers can be used for immobilization onto DNA capture

beads as shown below under "One Fragment = One Bead".



Library Preparation

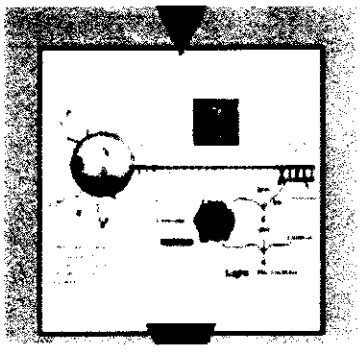
Using a series of standard molecular biology techniques, short adaptors (A and B) - specific for both the 3' and 5' ends - are added to each fragment. The adaptors are used for purification, amplification, and sequencing steps. Single-stranded fragments with A and B adaptors compose the sample library used for subsequent workflow steps.

One Fragment = One Bead

The single-stranded DNA library is immobilized onto specifically designed DNA Capture Beads. Each bead carries a unique single-stranded DNA library fragment. The bead-bound library is emulsified with amplification reagents in a water-in-oil mixture resulting in microreactors containing just one bead with one unique sample-library fragment.

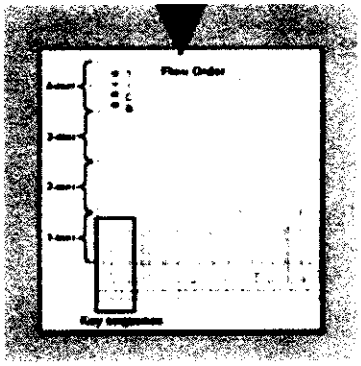
emPCR (Emulsion PCR) Amplification

Each unique sample library fragment is amplified within its own microreactor, excluding competing or contaminating sequences. Amplification of the entire fragment collection is done in parallel; for each fragment, this results in a copy number of several million per bead. Subsequently, the emulsion PCR is broken while the amplified fragments remain bound to their specific beads.



One Bead = One Read

The clonally amplified fragments are enriched and loaded onto a PicoTiterPlate device for sequencing. The diameter of the PicoTiterPlate wells allows for only one bead per well. After addition of sequencing enzymes, the fluidics subsystem of the Genome Sequencer FLX Instrument flows individual nucleotides in a fixed order across the hundreds of thousands of wells containing one bead each. Addition of one (or more) nucleotide(s) complementary to the template strand results in a chemiluminescent signal recorded by the CCD camera of the Genome Sequencer FLX Instrument.



Data Analysis

The combination of signal intensity and positional information generated across the PicoTiterPlate device allows the software to determine the sequence of more than 400,000 individual reads per 7.5-hour instrument run simultaneously. For sequencing-data analysis, three different bioinformatics tools are available supporting the following applications: de novo assembly up to 120 megabases; resequencing up to 3 gigabases; and amplicon variant detection by comparison with a known reference sequence.

3.3. Selection, Randomization Labeling or Blinding Procedure: Respiratory samples and the questionnaire will be labeled with a label (Appendix 2) that contains a barcode and a randomly generated number that will be linked to the questionnaire as well as the sample. The number consists of the following: "ABCDEFGVWXYZN (or T); "ABCD" is the WRAIR protocol number, "EFG" is the country code (Appendix 5), "VWXYZ" is the sample number, "N" or "T" is the sample type (throat or nasal). The label and the barcode will not contain any personal identifying information.

3.4. Discontinuation Criteria: Volunteers who are unable to provide at least one respiratory specimen will be discontinued.

3.5. Maintenance of Codes: The samples will be linked to the questionnaires by the barcode label, which will be maintained by an individual in the individual Embassy clinic not directly related to the study. This link will be maintained at the Embassy clinic for a period of 6 months, afterwhich it will be destroyed. WRAIR investigators will not have access to this link at any time.

3.6. Source Data to be Recorded Directly on the Case Report Forms (CRFs): See Questionnaire (Appendix 3). This questionnaire will be linked to the respiratory sample collected by the label (see Section 3.3), but will contain no personal identifying information.

3.7. Disposition of Data: Data, to include questionaires, and electronic databases will be maintained in a secure location at CMU, DVD, WRAIR.

4. Selection and Withdrawal of Subjects: Inclusion Criteria include: 1) Male or female between newborn and 65 years of age; 2) English understanding; 3) Have an acute respiratory disease; 4)Able to provide at least one respiratory specimen; 5) Eligible for care at the US Embassy health care facility. Volunteers who are unable to provide at least one respiratory specimen will be withdrawn.

5. **Calculations and Statistics:** No formal hypothesis will be tested. Descriptive statistics will be used to summarize the aggregate results, the results within each site and to compare the different sites.

6. **Direct Access to Source Data for Monitoring or Review by WRAIR IRB:** Data will be available for review by the WRAIR IRB and the representatives of the U.S. Department of Defense and Department of State.

7. **Protocol Deviation and Violations:** The approved protocol shall be conducted as described. On occurrence of a protocol deviation, a log will be kept by the study site, and reported to the WRAIR IRB. Each study site will notify the PI of any deviations weekly. Any deviation that impacts the scientific integrity of the study or that impacts the safety, rights and welfare of the subject will be promptly reported to the WRAIR IRB within 10 working days from knowledge of the event.

8. **Protocol Amendments:** All changes to the protocol will be submitted to the local IRB of record for approval. No amendments to this protocol will be made without the approval of the local IRB of record.

9. **Ethics and Informed Consent:** As this is a surveillance study using coded specimens, informed consent will not be obtained. Subjects will be provided with an information sheet.

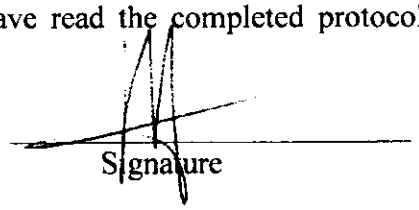
10. **Confidentiality and IRB approval:** Established procedures will be utilized to ensure confidentiality is maintained. Questionnaire documents, after completion by the subject, will be kept on-site in locked, secure locations, until shipment to the WRAIR. These documents will be shipped by traceable courier with the corresponding clinical specimens at a frequency to be determined according to sample acquisition frequency for that particular site. Once received at WRAIR, the questionnaires will be QA'd to ensure correct completeness, and to ensure there is a questionnaire for each subject's specimen(s) shipped. At the WRAIR, questionnaires will be kept in a secure location; investigators will have access to these documents. Results will be collected and reported to the Embassy sites on a periodic and aggregate basis (i.e., monthly, quarterly), depending on the frequency of cases seen and specimen shipments received from the individual Embassy sites.

11. **Unanticipated Problems**: An unanticipated problem involving risks to subjects or others is one that does not appear as a risk in the protocol.
    - **11.1.** Collecting Unanticipated Problems: The Principal Investigator will be notified immediately if he is not present when the study staff learns of an unanticipated problem in which protocol execution has put subjects or others at a risk. This can be done by email or telephone.
    - **11.2.** Reporting Unanticipated Problems: Reports of unanticipated problems will be promptly forwarded to the Walter Reed Army Institute of Research (WRAIR)

(DHSP), WRAIR. This report will be by phone (301) 319-9940 or by fax (301) 319-9961. A written report will then be completed and sent by email (WRAIRDHSP@amedd.army.mil). Investigators will also notify the local institutions per the respective institutions' guidelines.

11.3. Compliance Inspections: The PI will immediately report to the WRAIR DHSP knowledge of any pending compliance inspection by any outside governmental agency.

12. Signature of the Principal Investigator: I have read the completed protocol and agree to conduct the study as outlined herein.



Arthur Lyons
LTC, MC
Department of Virus Diseases
Walter Reed Army Institute of Research

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13.3. Dunbar SA. 2006. Applications of Luminex xMAP technology for rapid high-throughput multiplexed nucleic acid detection. Clin. Chim. Acta **363:** 71-82.

13.4. Sung, H., et al., 2008. [Evaluation of Seeplex(TM)RV Detection Kit for Detecting Rhinovirus, Human Metapneumovirus, and Coronavirus.]. Korean J Lab Med, **28**(2): p. 109-17.

13.5. Roh, K.H., et al., 2008. Comparison of the Seeplex reverse transcription PCR assay with the R-mix viral culture and immunofluorescence techniques for detection of eight respiratory viruses. Ann Clin Lab Sci, **38**(1): p. 41-6.

13.6. Yoo, S.J., E.Y. Kuak, and B.M. Shin, 2007. Detection of 12 respiratory viruses with two-set multiplex reverse transcriptase-PCR assay using a dual priming oligonucleotide system. Korean J Lab Med, **27**(6): p. 420-7.

14. Appendix 1: List of Abbreviations

ADV: Adenovirus
AFEB: Armed Forces Epidemiology Board
AI: Avian Influenza
AMEDD: Army Medical Department
ATCC: American Type Culture Collection
BCT: Basic Combat Training
BTC: Basic Training Center
CDC: Centers for Disease Control
CFR: Code of Federal Regulations
CMU: Compliance Management Unit, WRAIR DVD
CONUS: Continental United States

CMU: Compliance Management Unit, WRAIR DVD
CONUS: Continental United States
CPE: Cytopathic Effect
CRF: Case Report Form
DHSP: Division of Human Subjects Protection
DNA: Deoxyribonucleic Acid
DoD: Department of Defense
DoS: Department of State
DVD: Division of Viral Disease
EST: Expressed Sequence Tag
EXO: Exonuclease
FSHP: Foreign Service Health Practitioner
FSRMO: Foreign Service Regional Medical Officer
GEIS: Global Emerging Infections Surveillance and Response System
GCP: Good Clinical Practices
HURC: Human Use Research Committee
IATA: International Air Transport Association
ICF: Informed Consent Form
ILI: Influenza Like Illness
IRB: Institutional Review Board
LAFB: Lackland Air Force Base
LES: Locally Engaged Staff
MRMC: Medical Research and Materiel Command
Nab: Neutralizing Antibody
NIH: National Institutes of Health
OD: Optical Density
PCR: Polymerase Chain Reaction
PI: Pandemic Influenza
RMO: Regional Medical Officer
RPM: Resequencing Pathogen Microarray
RT-PCR: Reverse Transcriptase Polymerase Chain Reaction
RVP: Respiratory Viral Panel
SAP: Shrimp Alkaline Phosphatase
SOP: Standard Operating Procedure
TBD: To be determined
TSP: Target Specific Primer
TSPE: Target Specific Primer Extension
USAF: United States Air Force
USAFSAM: United States Air Force School of Aerospace Medicine
VTM: Viral Transport Media
WHO: World Health Organization
WRAIR: Walter Reed Army Institute of Research
WRAMC: Walter Reed Army Medical Center

17. Appendix 2: Labels

18. Appendix 3: Respiratory Illness Questionnaire/Instruction Sheet **Barcode :**

US Embassy/Consulate Location:_____ Date of Encounter:_____

Volunteer Gender: _____ Ethnicity: _____

Highest Oral Temperature (if known):_____ Time/Date taken: _____

Circle the symptoms experienced: Sore throat Cough Chills Chest Pain Shortness of Breath Body Aches Fatigue Feverish Headache Runny Nose Congestion Diarrhea Vomiting
When did symptoms start:

Travel: Where has the patient been in the last 2 weeks: _____

Did the patient receive the flu shot this year:_____

If yes, When: _____

☐ **I have provided the information sheet to the volunteer.**

☐ **The patient agrees to the future use of his/her samples.**

Directions for Specimen Collection:

Open Kit and remove patient questionnaire.
Have patient complete questionnaire.
Have patient read Informational Document section.
Check the box "I have provided the information below to the patient" and "The patient agrees to the future use of the samples provided".
Remove smaller swab first from packaging.
(Warning: Do Not insert swab into solution before collecting patient specimen.)
Insert small swab into nostril, 3 inches deep.
Rotate swab slowly one full turn.

Remove swab from nose.
Place swab into tube labeled "Nasal"
Break off excess swab handle at scored mark.
Cap and close tube tightly, set aside.
10) Remove larger swab from packaging.
11) Vigorously swab posterior pharynx, tonsils and adenoids .
Remove swab from throat area.
Place swab into tube labeled "Throat".
Break off excess swab handle at scored mark.
Cap and close tube tightly.
Place completed questionnaire and 2 tubes in Ziploc bag.
Seal bag closed.
Store until ready to ship.

For Surveillance Use Only Samples can be stored at ambient temperature.

PrimeStore Reagent Safety and Health Precautions

PrimeStore contains a proprietary blend of reagents that includes a reducing and chaotrophic compound (Health Rating: 1, Reactivity: 1). These chemicals can be harmful if inhaled and/or ingested. PrimeStore may cause irritation to skin, respiratory tract and eyes. If PrimeStore comes into direct contact with skin or eyes wash with copious amounts of water for 5 minutes. Seek medical advice if irritation persists. Collect clinical sample on dry swab, place into PrimeStore tube and ensure cap is tightly sealed.

First Aid Measures	
Eye Contact	Immediately flush eyes, occasionally lifting the upper and lower eyelids. Get medical aid immediately.
Ingestion	Do not induce vomiting. If victim is conscious and alert, give 2-3 cupfuls of water. Never give anything by mouth to an unconscious person get medical aid immediately.

19. Appendix 4 :Information Sheet

INFORMATION SHEET

TITLE: Surveillance of Acute Infectious Respiratory Disease in Select Populations: US Embassy/Consulate Employees

You and/or your child are being asked to participate in a surveillance study conducted at the US Embassy/Consulate located in (insert name of city/country here) by John Keyes, M.D., M/MED/DASHO and Arthur Lyons, M.D., Ph.D., Dept of Defense (US Army). Participation in this study is entirely voluntary. You should read the information below, and ask questions about anything you do not understand. You will receive a copy of this information sheet.

Purpose of the Study

The purpose of this surveillance study is to determine the causes of respiratory infections in people who work or visit US Embassies worldwide. Surveillance is the process of learning about different types of infections to help prevent and control diseases. The results of the study will help us better understand the causes of respiratory illness in people who work in and visit US Embassies around the world and may help us devise ways to prevent some of these causes in the future.

Procedures

As part of this study, we will ask you and/or your child to do the following things:

a. Allow us to take one swab of the back of your and/or your child's throat and one swab of the inside of your and/or your child's nose.
b. Fill out a very brief questionnaire about your and/or your child's current illness.

The leftover throat or nasal samples that you and/or your child are donating under this study could be useful in other health research studies about different types of infectious diseases. If you give your permission to use your and/or your child's leftover sample in future health research studies, you will not receive any notice of future uses of your and/or your child's sample(s). The samples collected today will be sent to the United States for storage and additional testing.

Potential Risks and Discomforts

The swabs may cause some brief discomfort that will go away after the swab is removed.

Confidentiality

The case records from this study will be available for review by members of the Institutional Review Board (IRB) at the Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD the Department of Defense, the Department of State and M/MED/DASHO as part of their normal duties. All study records will be kept in a confidential form. Only the physicians conducting this study will have access to the records from this study. Information gained from this study may be used as part of a scientific publication, but you and/or your child will not be personally identified. A code will be used to identify your study records and samples. The code will not contain information that could identify you, and will be stored in a safe place.

Questions about the study
If you have questions regarding the study itself, please contact the Embassy/Consulate personnel at the number below. If you have questions about your and or your child's rights, please contact the Director, WRAIR Division of Human Subjects Protection, 503 Robert Grant Ave., Silver Spring, MD 20910, (301) 319-9940.

Dr. John Keyes (202) 663-1518
Embassy/ Consulate Contact: (Name, telephone number)

22. Appendix 5: Proposed List of US Embassy Sites and Country Codes

001. Lagos, Nigeria
002. Dakar, Senegal
003. Cairo, Egypt
004. Frankfurt, Germany
005. Moscow, Russia
006. New Delhi, India
007. Jakarta, Indonesia
008. Beijing, China
009. Mexico City, Mexico
010. Amman, Jordan
011. Baku, Azerbaijan
012. Baghdad, Iraq
013. Kabul, Afghanistan
014. Bogota, Colombia
015. La Paz, Bolivia
016. Brasilia, Brazil
017. Hong Kong, China
018. Kuala Lumpur, Malaysia
019. Manila, Philippines
020. Seoul, S. Korea
021. Phnom Penh, Cambodia
022. Vientiane, Laos
023. Ankara, Turkey
024. San Salvador, El Salvador
025. Singapore, Singapore
026. Taipei, Taiwan
027. Tel Aviv, Israel
028. Bangkok, Thailand
029. Katmandu, Nepal
030. Ho Chi Minh City, Vietnam
031. Hanoi, Vietnam
032. Rangoon, Burma
033. Ulaanbaatar, Mongolia